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B. White
9-20-94

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Applicants: | Stavrianopoulos et al. ) | |
| Serial No.: | 07/967,646 ) | Group Art Unit: 1807 |
| Filing Date: | October 28, 1992 ) | Exam'r: Ardin Marschel, Ph.D |
| For: | COMPOSITION AND KIT EMPLOYING ) | |
| | EMPLOYING CHEMICALLY LABELLED ) | |
| | POLYNUCLEOTIDE PROBES (As Amended) ) | |

575 Fifth Avenue (18th Foor)
New York, New York 10017

Honorable Commissioner
of Patents & Trademarks
Washington, D.C. 20231

**DECLARATION IN SUPPORT OF UTILITY**
**OF DR. DEAN L. ENGELHARDT**

I, Dean L.Engelhardt, hereby declare as follows:

1. I am currently employed by Enzo Biochem, Inc., 575 Fifth Avenue (18th Floor), New York, New York 10017 as Senior Vice President, having held that position since 1988. Prior to my employment at Enzo Biochem, Inc., I was Associate Professor of Microbiology at Columbia University College of Physicians and Surgeons, New York City, having obtained my doctorate from Rockefeller University in New York City.

3. I am familiar with the contents of the above-identified application including the pending claims 27-47, as well as the original disclosure represented by U.S. Patent Application Serial No. 06/491,469, filed on January 27, 1983. I have reviewed both the July 25, 1993 Office Action and the earlier October 26, 1993 Office Action issued in connection with the subject application. I understand from the July 25, 1994 Office Action that the Examiner has taken the position that:

> Claims 27-45 are rejected under 35 U.S.C. §101 because there is no instantly disclosed utility for the double-stranded containing composition. This rejection is reiterated and maintained as set forth in the previous office action mailed 10/22/93. Arguments argue that the double-stranded form is an intermediate prior to detection. This is non-persuasive in that this utility has not been found in the disclosure as filed. Adding it at this time does not support the utility of the invention as disclosed. Also applicants argue that double-stranded form passes the strictures of utility irrespective of whether detection has been carried out. This is confusing and non-persuasive because the Examiner is unaware of such an inherent utility in double-stranded nucleic acid.

4. I also understand that in the October 26, 1993 Office Action the then-pending claims 27-45 were also rejected under 35 U.S.C. §101 "because there is no instantly disclosed utility for a double stranded containing composition. It is acknowledged that probes that are single stranded have utility as probes but after a double stranded form has been produced as cited in claims 27-45, it no longer has a utility as for detection."

5. I am submitting this Declaration in support of the utility of the subject matter set forth in pending claims 27-45.

6. In addition to my position as Senior Vice President of Enzo Biochem, Inc., I have also served as Director of Research in which I have overseen scientific research activities. Among my responsibilites at Enzo Biochem have been the development of new nucleic acid technology and hybridization formats, including new diagnostic and therapeutic approaches and agents based upon nucleic acid technology. Accordingly, I am quite familiar with the technology relating to this application and the pending claims.

7. As set forth in claim 27, the broadest composition claim, the instant composition comprises a transparent non-porous or translucent non-porous system further comprising a double-stranded oligonucleotide or polynucleotide which is directly or indirectly fixed or immobilized to a solid support wherein one of the strands comprises a chemical label that further comprises a signalling moiety which is capable of generating a soluble signal. Other embodiments or aspects of the invention define the solid support (claims 28-31), the system (claims 32-33), the solid support and the system (claims 34 and 35), the fixation or immobilization to the solid support (claims 36 and 37), the attachment of the signalling

moiety (claims 38-40), the attachment of the chemical label (claims 41 and 42), the signalling moiety (claim 43), and the soluble signal (claims 44 and 45).

8. The instant double-stranded composition is useful in carrying out the assay disclosed in the specification (see, for example, the section in the specification titled "SUMMARY OF THE INVENTION," beginning on page 21, line 15, through page 22, lines 9+) which is also the subject of the method claims in related U.S. Patent No. 4,994,373.

9. More specifically, a double-stranded composition is formed, such as defined in claim 27, where the chemically labelled strand is itself immobilized (see the specification, for example, page 26, second paragraph; and page 29, lines 1-3 and lines 21-24), or where the chemically labelled strand becomes immobilized through its specific recognition of the sequence in the other strand fixed or immobilized to the solid support (see the specification, for example, page 21, line 27, through page 22, line 9; page 27, last paragraph; and page 30, second paragraph). The hybridized signal carrying strand is distinguished from any unhybridized signal carrying strands to provide qualitative detection or quantitative determination of a nucleic acid or genetic material of interest. If, for example, the signal carrying strand in solution hybridizes to a strand already fixed or immobilized to a solid support, then an obvious and altogether conventional procedure such as washing is performed to rid the assay system of the single-stranded signal carrying strand in solution (see specification, for example, page 27, last paragraph, particularly lines 29-31). The detection of the soluble signal (the label not washed out) in such an instance is a measure of the soluble signal present in the double-stranded oligo- or polynucleotide. If, on the other hand, for example, the signal carrying strand is itself fixed or immobilized to the solid support, single strands can be conventionally and selectively destroyed through obvious and conventional procedures such as enzymatic digestion using for example, an S1 nuclease, or an S1 nuclease in conjunction with other nucleases, e.g., exonuclease I. [See, for example, column 20, lines 25-35 in Engelhardt et al., U.S. Patent No. 5,241,060, issued on August 31, 1993 (copy attached hereto as Exhibit I).✓ This patent issued from an application related to U.S. Patent Application Serial No. 06/391,440, filed on June 23, 1982. The aforementioned Serial No. 06/391,440 is referenced in the instant specification on page 13, lines 1-3; and also on page 21, second paragraph, the latter incorporating Serial No. 06/391,440 by reference into the instant specification. The remaining label will be a valid measure of the unlabelled strand because the

unhybridized single strands (labelled or unlabelled) will have been eliminated. Therefore, from the double-stranded composition formed intermediately, useful and even crucial functions and analyses can be performed in accordance with the instant disclosure. The utility described in this paragraph is obvious from the instant disclosure, and would have been obvious to any person familiar with the technology and subject matter to which the instantly claimed invention pertains.

10. In order to perform the assay referred to in Paragraph 8 above and illustratively described in the preceding paragraph, a double-stranded composition must be formed if the nucleic acid or genetic material of interest is present. The double strand results from the recognition and hybridization of the two oligonucleotide or polynucleotide strands, one strand being unlabelled and the other comprising a chemical label further comprising a signalling moiety capable of generating a soluble signal. To state it differently, the double-stranded composition is *sine qua non* in the differentiation between reacted (hybridized) and unreacted (unhybridized) labelled oligonucleotide or polynucleotide.

Thus, the double-stranded oligonucleotide or polynucleotide in the instant composition is useful in carrying out the patented assay method set forth in U.S. Patent No. 4,994,373, and this utility is obvious from the present disclosure, if not explicitly stated in the specification.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Sept 2, 1994
Date

Dean L. Engelhardt
Dean L. Engelhardt

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US005241060A

# United States Patent [19]

Engelhardt et al.

[11] Patent Number: 5,241,060

[45] Date of Patent: Aug. 31, 1993

[54] **BASE MOIETY-LABELED DETECTABLE NUCLEATIDE**

[75] Inventors: **Dean Engelhardt; Elazar Rabbani,** both of New York; **Stanley Kline,** Brooklyn; **Jannis G. Stavrianopoulos,** New York; **Dollie Kirtikar,** Elmhurst, all of N.Y.

[73] Assignee: **Enzo Diagnostics, Inc.,** Farmingdale, N.Y.

[21] Appl. No.: **532,704**

[22] Filed: **Jun. 4, 1990**

**Related U.S. Application Data**

[60] Division of Ser. No. 140,980, Jan. 5, 1988, abandoned, which is a continuation of Ser. No. 674,352, Nov. 21, 1984, abandoned, which is a continuation of Ser. No. 391,440, Jun. 23, 1982, abandoned.

[51] **Int. Cl.**[5] ..... **C07H 15/12**

[52] **U.S. Cl.** ..... **536/27**

[58] **Field of Search** ..... 536/27

[56] **References Cited**

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*Primary Examiner*—John W. Rollins
*Attorney, Agent, or Firm*—Ronald C. Fedus

[57] **ABSTRACT**

The present invention provides nucleotides and polynucleotides which are chemically modified or labeled so as to be capable of ready detection when attached to and/or incorporated in nucleic acid material. More particularly, this invention provides a nucleotide having the formula

PM-SM-BASE-Sig

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a pyrimidine, purine or 7-deazapurine moiety. PM is attached at the 3' or the 5' position of SM when the nucleotide is a deoxyribonucleotide and at the 2', 3' or 5' position when the nucleotide is a ribonucleotide. BASE is attached to the 1' position of SM from the $N^1$ position when BASE is a pyrimidine or the $N^9$ position when BASE is a purine or a 7-deazapurine. Sig is a detectable moiety that is covalently attached to BASE at a position other than the $C^5$ position when BASE is a pyrimidine, at a position other than the $C^8$ position when BASE is a purine and at a position other than the $C^7$ position when BASE is a 7-deazapurine.

**31 Claims, 4 Drawing Sheets**